REMARKS

The Invention.

This invention provides for methods of obtaining proteins which induce a lower allergenic response in humans exposed to the proteins. Specifically, T-cell epitopes of a precursor protein are altered to produce a protein of lowered allergenicity.

Status of the Application.

Claims 1-16 are pending with claims 1-12 and 15 and 16 being withdrawn from consideration. The amendment to claim 13 clarifies how a T cell epitope is to be determined. It merely incorporates the assay element of claim 12 (now claim 17). Support for adherent monocytes can be found in the specification as filed on page 25, line 32. New claim 17 is the same as claim 12 of the original application. No new matter has been added by these amendments.

Claims 13, 14 and 17 (original claim 12) stand rejected under 35 U.S.C. § 103(a) as allegedly obvious.

Interview with Examiner Saunders.

The Applicants and Manley Huang, Ph.D. would like to thank Examiner Saunders for his time and graciousness in listening to their arguments in a personal interview on March 6, 2001. The following remarks incorporate many aspects of that discussion.

New claim 17.

New claim 17 appears in this Request for Reconsideration. Claim 17 is identical to claim 12 of the originally filed application as well as its divisional application 09/255,055 as amended in paper 10 (Amendment and Request for Reconsideration mailed June 16, 2000. USSN 09/255,055 has been abandoned.

During a personal interview with Examiner Saunders, it was suggested that, because the remaining issues in the prosecution of claims 13, 14 and 12 are the same, that they be recombined in this application. See Paper 22 (Interview Summary).

Sequence Listing.

The Applicants have filed, with this Request for Reconsideration, a new Sequence Listing that contains SEQ ID NO's for all the individual peptides. If appropriate, the Applicants are willing to provide a substitute specification wherein peptide sequences are identified by SEQ ID NO's.

35 U.S.C. §103.

Claims 13 and 14 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over US Patent 5,820,862, Bhardwaj, *et al., J. Clin. Invest.* **94**:797 (1994) and US Patent 5,648,219.

In considering obviousness, the prior art as a whole must be considered and its teachings must be viewed as they would have been by one of skill in the art at the time of the invention. To establish a prima facie case of obviousness, the Examiner must cite prior art that discloses each element of the claims unless the element would be obvious to one of skill in the art. The Examiner must also provide reasons or motivation for one of skill to combine the prior art references to carry out the claimed method and demonstrate that one of ordinary skill would have had a reasonable expectation of success in attempting in carrying out the method. *In re Vaeck*, 20 USPQ 2d 1438 (Fed. Cir. 1991).

The Examiner states the Bhardwaj reference teaches the obtaining of CD4+ and CD8+ T cells from naïve individuals. However, on page 799, second column, last sentence of the second paragraph, Bhardwaj, *et al.* state:

> Because CTL activity was measured on influenza A/PR8-infected targets, a strain first identified in 1934, and the prevalent strains are A/Texas/36/91 and A/Beijing/232/92, ***the CTLs generated appear to be cross-reactive***, confirming other studies of human influenza-specific CTLs. (*emphasis added)*

This cross-reactivity indicates the donors were sensitized to influenza and were, in fact, not naïve.

Thus, none of the references cited by the Examiner recites the use of naïve donors as a source of T-cells for determining immunogenic T-cell epitopes. Furthermore, it would not have been obvious to one of skill in the art to use T-cells from a naïve donor as one of skill would not have had a reasonable expectation of success in doing so.

Schlienger, *et al., Blood* **96**:3490 (2000), an after-filing reference, indicates the belief of those of skill in the art. Schlienger, *et al.* report that although "monocyte-derived DCs [dendritic cells] have been utilized to sensitize total CD4+ T cells *in vitro*, only ***secondary proliferation to neoAgs could be elicited***." Schlienger, *et al., Blood* **96**:3490 (2000) (see abstract; emphasis added). The authors also state: "Furthermore, the induction of Ag-specific proliferation from naïve precursors has to date been described with mature DC's isolated directly from peripheral blood." *Id.*, Introduction, second paragraph. Thus, until June 23, 1999 (the date the Schlienger manuscript was accepted for publication), it was believed by those in the art that monocyte derived dendritic cells could be used to stimulate only secondary responses in naïve T-cells.

The Schlienger reference contains citations to other studies that formed the basis of their conclusion. Macatonia, *et al., Immunol.* 74:399 (1991) described experiments in which they generated a primary proliferative T-cell response to HIV and HIV peptides *in vitro*. However, mature dendritic cells were used. No proliferation was seen when adherent monocytes were used as antigen presenting cells (*Id.* at page 404, first column). In a similar vein, Mehta-Damani, *et al., Eur. J. Immunol.* **25**:1206 (1995) used dendritic cells to generate CD4+ T cell lines from naïve precursors. They found proliferation when dendritic cells presented keyhole limpet hemocyanin, sperm whale myoglobin and HIV gp160. Macrophages did not stimulate naïve T cell proliferation.

From the same laboratory, Takamizawa, *et al., J. Immunol.* **158**:2134 (1997) isolated CD2+ dendritic cell precursors and measured the proliferative response by naïve T-cells to these antigen presenting cells. However, before isolating the dendritic cell precursors from peripheral blood, the authors depleted the PBMC of adherent

monocytes, the claimed source of dendritic cells in the instant invention. As had been demonstrated in earlier studies, adherent monocytes did not present protein antigens to naïve T-cells.

In a study with dendritic cell precursors, Zhou and Tedder (USP 5,849,589 and *Proc. Nat'l Acad. Sci. USA* **93**:2588 (1996) used plastic adherent monocytic cells as a source of dendritic cells. However, they used the differentiated dendritic cells to stimulate a mixed lymphocyte response. As is well known in the art, alloreactive T cells in a MLR will proliferate regardless of antigen presented (see Janeway, *et al.*, enclosed) and therefore although Zhou and Tedder showed that plastic adherent monocytes could differentiate into mature dendritic cells, they did not show that cells so obtained could successfully present antigen.

Because the use of naïve T-cells and dendritic cells derived from adherent monocytes were not disclosed in prior art references and in fact, until 1999 (at least) monocyte-derived dendritic cells were believed to generate only secondary responses (see Schleinger), it would not have been obvious to one of skill to use naïve T-cells in the claimed methods.

Claim 17 (original claim 12 in USSN 09/255,505) stands rejected under 35 USC §103(a) as allegedly obvious over Worthington, *et al.*, in view of Bhardwaj, *et al.*, Schuler, *et al.* and Mackay, *et al.* The Examiner states that Mackay, *et al.* "teach that dendritic cells can present antigen to naïve T-cells" and therefore it would have been obvious to use the dendritic cells suggested by Mackay, *et al.* in the assays of Worthington, *et al.*

The Applicants respectfully disagree. The naïve T-cells described by Mackay were used only in a mixed lymphocyte reaction (see Example VII). They never described the use of naïve T-cells to generate a primary response to dendritic cells primed with antigen. Thus, this work does not negate the finding in 1999 that monocyte derived dendritic cells could elicit only a secondary response from naïve T-cells (see Schleinger).

In light of the above remarks, the Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-7609.

Respectfully submitted,

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Appendix I

13. (Once amended)A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell with a peptide comprising said T-cell epitope; and

(ii) contacting said dendritic cell and peptide to a naïve T cell; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T cells [in a sample].

14. The method according to claim 13, wherein said epitope is modified by:

(a) substituting the amino acid sequence of the epitope with an analogous sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of the epitope with an analogous sequence from a non-human homolog to the protein of interest, which analogous sequence produces a lesser allergenic response from T-cells than that of the protein of interest; or

(c) substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope, but which produces a lesser allergenic response from T cells than that of the protein of interest.

17. (New) A method for determining a T-cell epitope of a peptide comprising the steps of:

(a) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

(b) promoting differentiation in said solution of dendritic cells;

(c) combining said solution of differentiated dendritic cells and said naïve CD4+and/or CD8+ T-cells with the peptide, said peptide comprising said T-cell epitope; and

(d) measuring proliferation of said T-cells in said step (c).